

Serial No. 10/019,586
Supp. Amdt. Dated June 16, 2004

Patent Docket No: P1746R1

Amendments to the Claims:

Claims 1-3, 8, 9, 12, 13, 15-19, 22, 24, 26, 29, 40, 44, 49, and 56 are amended without prejudice or disclaimer.

Claims 59-101 are cancelled without prejudice or disclaimer.

New claims 102-103 are added.

This listing of claims will replace all prior versions, and listings, of claims in the application.

1 (currently amended): A polynucleotide comprising:

- (a) an amplifiable selectable gene;
- (b) a green fluorescent protein (GFP) gene; and
- (c) a selected sequence encoding a desired product, wherein the selected sequence is operably linked to either the amplifiable selectable gene or to the GFP gene, and to a promoter, and wherein the selected sequence and the promoter are (i) operably linked to the amplifiable selectable gene or (ii) operably linked to the GFP gene;
~~wherein the selected sequence encodes a protein selected from the group consisting of cytokines, lymphokines, enzymes, antibodies, and receptors.~~

2 (currently amended): The polynucleotide of claim 1, wherein the amplifiable selectable gene is selected from the group of consisting of the genes encoding dihydrofolate reductase (DHFR) and the gene encoding glutamine synthetase.

3 (currently amended): The polynucleotide of claim 2, wherein the amplifiable selectable gene is the dihydrofolate reductase (gene encoding DHFR) gene.

4 (original): The polynucleotide of claim 1, wherein the GFP gene encodes a mutant GFP.

5 (original): The polynucleotide of claim 4, wherein the mutant GFP exhibits a higher fluorescence intensity than the wild-type GFP.

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6 (original): The polynucleotide of claim 4, wherein the mutant GFP is GFP-S65T having a serine to threonine substitution in amino acid 65 of the wild-type GFP of *Aequorea victoria*.

7 (cancelled)

8 (currently amended): The polynucleotide of claim 1, wherein the amplifiable selectable gene is fused to the GFP gene ~~as to form a fusion gene, wherein the fusion gene is operably linked to the promoter.~~

9 (currently amended): The polynucleotide of claim 8, wherein the amplifiable selectable gene is the ~~DHFR~~ gene encoding DHFR.

10 (original): The polynucleotide of claim 8, further comprising an intron between the promoter and the selected sequence, the intron defined by a 5' splice donor site and a 3' splice acceptor site.

11 (original): The polynucleotide of claim 10, wherein the intron provides a splicing efficiency of at least 95%.

12 (currently amended): The polynucleotide of claim 10, wherein the fusion gene is positioned within the intron ~~and wherein the fusion gene and selected sequence are operably linked to the promoter 5' of the intron.~~

13 (currently amended): The polynucleotide of claim 10, further comprising an internal ribosome entry site (IRES) between the selected sequence and the fusion gene, ~~wherein the selected sequence and fusion gene are operably linked to the promoter 5' of the selected sequence.~~

14 (original): The polynucleotide of claim 1, further comprising 3' of the promoter: an intron defined by a 5' splice donor site and a 3' splice acceptor site providing a splicing efficiency of at least 95%; and an IRES; wherein the selected sequence is positioned between the intron and the IRES.

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15 (currently amended): The polynucleotide of claim 14, wherein the amplifiable selectable gene is positioned in the intron and the GFP gene is positioned 3' of the IRES, and wherein the amplifiable selectable gene is operably linked to the promoter.

16 (currently amended): The polynucleotide of claim 14, wherein the GFP gene is positioned in the intron and the amplifiable selectable gene is positioned 3' of the IRES, and wherein the GFP gene is operably linked to the promoter.

17 (currently amended): ~~The A~~ polynucleotide of ~~claim 1, further~~ comprising: a first transcription unit comprising a first promoter ~~followed by~~, an intron positioned 3' to the first promoter, and ~~the~~ a selected sequence positioned 3' to the intron; and a second transcription unit comprising a second promoter and an intron positioned 3' of the second promoter; wherein the intron in the first transcription unit is the first intron, and the intron in the second transcription unit is the second intron, and wherein each of the first and the second introns is defined by a 5' splice donor site and a 3' splice acceptor site providing a splicing efficiency of at least 95%, wherein the polynucleotide further comprises an amplifiable selectable gene and a GFP gene, and wherein the selected sequence and the promoter are (i) operably linked to the amplifiable selectable gene or (ii) operably linked to the GFP gene.

18 (currently amended): The polynucleotide of claim 17, wherein the amplifiable selectable gene is positioned in the first intron, ~~in the first transcription unit~~ wherein the amplifiable selectable gene and the selected sequence are both operably linked to the first promoter; and wherein the GFP gene is positioned 3' of the second intron and operably linked to the second promoter ~~in the second transcription unit.~~

19 (currently amended): The polynucleotide of claim 17, wherein the second transcription unit further comprises a selected sequence positioned 3' of the second intron, wherein the selected sequence in the first transcription unit is the first selected sequence, and the selected sequence in the second transcription unit is the second selected sequence, and wherein the second selected sequence is operably linked to the second promoter and encodes a second desired product.

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20 (original): The polynucleotide of claim 19, wherein the amplifiable selectable gene is positioned in the first intron and operably linked to the first promoter, and the GFP gene is positioned in the second intron and operably linked to the second promoter.

21 (original): The polynucleotide of claim 19, wherein the GFP gene is positioned in the first intron and operably linked to the first promoter, and the amplifiable selectable gene is positioned in the second intron and operably linked to the second promoter.

22 (currently amended): The polynucleotide of claim 19, further comprising an IRES positioned 3' of the second selected sequence.

23 (original): The polynucleotide of claim 22, wherein the amplifiable selectable gene is positioned in the first intron and operably linked to the first promoter, and the GFP gene is positioned 3' of the IRES and operably linked to the second promoter.

24 (currently amended): The polynucleotide of claim 19, wherein the amplifiable selectable gene is fused to the GFP gene to form a fusion gene ~~wherein the fusion gene is positioned in the first intron.~~

25 (original): The polynucleotide of claim 24, wherein the second transcription unit further comprises a selectable marker gene positioned in the second intron and operably linked to the second promoter.

26 (currently amended): The polynucleotide of claim 19, wherein the first transcription unit further comprises an IRES positioned 3' of the first selected sequence.

27 (original): The polynucleotide of claim 26, wherein the amplifiable selectable gene and the GFP gene are fused to form a fusion gene positioned 3' of the IRES and operably linked to the first promoter.

28 (original): The polynucleotide of claim 27, wherein the second transcription unit further comprises a selectable marker gene positioned in the second intron and operably linked to the second promoter.

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29 (currently amended): The polynucleotide of claim 26, wherein the second transcription unit further comprises an IRES positioned 3' of the second selected sequence, wherein the IRES in the first transcription unit is the first IRES, and the IRES in the second transcription unit is the second IRES.

30 (original): The polynucleotide of claim 29, wherein the amplifiable selectable gene is positioned 3' of the first IRES and operably linked to the first promoter, and GFP gene is positioned 3' of the second IRES and operably linked to the second promoter.

31 (original): The polynucleotide of claim 19 wherein the first promoter and the second promoter are the same type of promoter.

32 (original): The polynucleotide of claim 31, wherein the first promoter and the second promoter are the CMV or the SV40 promoter.

33 (original): The polynucleotide of claim 19, wherein at least one of the promoters is inducible.

34 (original): The polynucleotide of claim 1, wherein the promoter is the human cytomegalovirus immediate early (CMV) promoter.

35 (cancelled)

36 (original): The polynucleotide of claim 1, wherein the selected sequence encodes a protein selected from the group consisting of neuronotrophin-3, deoxyribonuclease, vascular endothelial growth factor, immunoglobulin and Her2 receptor.

37 (original): The polynucleotide of claim 19, wherein the first selected sequence encodes an immunoglobulin heavy chain and the second selected sequence encodes an immunoglobulin light chain.

38 (original): The polynucleotide of claim 19, wherein the first selected sequence encodes one polypeptide chain of a multichain receptor, and the second selected sequence encodes a second polypeptide chain of the receptor.

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39 (original): The polynucleotide of claim 1 that replicates in a eukaryotic host cell.

40 (currently amended): A polynucleotide comprising:

- a) an amplifiable selectable gene;
- b) a fluorescent protein gene; and
- c) a selected sequence encoding a desired product, wherein the selected sequence is

operably linked to either the amplifiable selectable gene or to the GFP gene, and to a promoter, and wherein the selected sequence and the promoter are (i) operably linked to the amplifiable selectable gene or (ii) operably linked to the fluorescent protein gene.

41 (original): A host cell comprising the polynucleotide of claim 1.

42 (original): The host cell of claim 41, wherein the cell is a mammalian cell.

43 (original): The host cell of claim 42 wherein the mammalian cell is a Chinese Hamster Ovary (CHO) cell.

44 (currently amended): The host cell of claim 43, wherein the amplifiable selectable gene is the gene encoding DHFR gene and the CHO cell has a DHFR- phenotype.

45 (original): The host cell of claim 43, wherein the desired product is selected from the group consisting of neuronotrophin-3, deoxyribonuclease, vascular endothelial growth factor, immunoglobulin and Her2 receptor.

46 (original): A kit comprising a container containing the polynucleotide of claim 1.

47 (original): A method of producing a desired product comprising introducing the polynucleotide of claim 1 into a suitable eukaryotic cell, culturing the resultant eukaryotic cell under conditions so as to express the desired product, and recovering the desired product.

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48 (original): The method of claim 47 wherein the desired product is recovered from the culture medium.

49 (currently amended): A method of obtaining a cell expressing a desired product, the method comprising:

- a) introducing the polynucleotide of claim 1 into a population of eukaryotic cells; and
- b) isolating the cells of step a) that express the green fluorescent protein gene and the amplifiable selectable gene, expression indicative of the cell also expressing the desired product.

50 (original): The method of claim 49, wherein the step of isolating cells expressing the green fluorescent protein gene comprises sorting for and cloning the brightest 1%-10% of fluorescent cells, wherein the sorting and cloning are performed using a fluorescence activated cell sorter.

51 (original): The method of claim 50, wherein the cells are subjected to two or more rounds of sorting, wherein the cells are cultured for a period of time between each round.

52 (original): The method of claim 51, wherein the cells are cultured for about two weeks between each round of sorting.

53 (original): The method of claim 52, wherein the cells are cultured in selection medium.

54 (original): The method of claim 52, wherein the brightest fluorescent cells are cultured in selection medium comprising an appropriate amplifying agent.

55 (original): The method of claim 52, wherein the brightest fluorescent cells are cultured in medium containing incremental amounts of the amplifying agent.

56 (currently amended): The method of claim 53, wherein the amplifiable selectable gene is the gene encoding DHFR and the amplifying agent is methotrexate.

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57 (original): The method of claim 54, further comprising analyzing the cells after culture with amplifying agent, for expression of the desired product to isolate cells producing high levels of the desired product.

58 (original): The method of claim 57, wherein the cells are analyzed for RNA encoding the desired product by RT-PCR, the amount of RNA indicative of the level of production of the desired product.

59-101 (cancelled)

102 (new): The polynucleotide of claim 1, wherein the GFP gene is a GFP-fusion gene.

103 (new): The polynucleotide of claim 1, wherein the selected sequence encodes a protein selected from the group consisting of cytokines, lymphokines, enzymes, antibodies, and receptors.